CR 92.004

June 1992

NCEL

Contract Report

An Investigation Conducted by
Mick F. Arthur, G. Kelly O'Brien,
Sarah S. Marsh, and Thomas C. Zwick
BATTELLE
Columbus Division

EVALUATION OF INNOVATIVE APPROACHES TO STIMULATE DEGRADATION OF JET FUELS IN SUBSOILS AND GROUNDWATER

Abstract The objective of this study was to evaluate the feasibility of surfactant-enhanced biodegradation of JP-5 in soil from Patuxtent Naval Air Test Center (NATC) under simulated conditions of soil venting. Surfactants and emulsifiers were screened for microbial toxicity and for their capacity to solubilize jet fuel from soil. Three surfactants were subsequently evaluated in 60-day flask aerobic biodegradation experiments. One surfactant was tested in soil columns under simulated soil venting conditions for 47 days. The results of the soil column study showed that the surfactant plus soil venting failed to enhance biodegradation of JP-5 compared to soil venting alone. Soil venting appears to overcome oxygen limitations in unsaturated soil and should be considered for enhanced biodegradation and soil bioremediation at NATC.

NAVAL CIVIL ENGINEERING LABORATORY PORT HUENEME CALIFORNIA 93043-5003

METRIC CONVERSION FACTORS

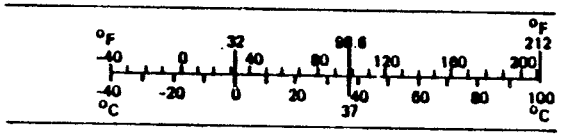
Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
		AREA		
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
		MASS (weight)		
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2,000 lb)	0.9	tonnes	t
		VOLUME		
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in. = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.

Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
		AREA		
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
		MASS (weight)		
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1,000 kg)	1.1	short tons	
		VOLUME		
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
		TEMPERATURE (exact)		
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-018

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1992	Final; June 1988 - August 1989

4. TITLE AND SUBTITLE
EVALUATION OF INNOVATIVE APPROACHES TO STIMULATE DEGRADATON OF JET FUELS IN SUBSOILS AND GROUNDWATER

5. FUNDING NUMBERS
PR - 71-033
C - DAAL03-86-D-0001
WU - DN668034

6. AUTHOR(S)
Mick F. Arthur, G. Kelly O'Brien, Sarah S. Marsh, and Thomas C. Zwick

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
BATTELLE
Columbus Division
505 King Avenue
Columbus, Ohio 43210-2693

8. PERFORMING ORGANIZATION REPORT NUMBER
CR 92.004

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Naval Facilities Engineering Command / Naval Civil Engineering Laboratory
Chesapeake Division
Washington Navy Yard
Washington, DC 20374-2121
Code L71
Fort Hueneme, CA 93043

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The objective of this study was to evaluate the feasibility of surfactant-enhanced biodegradation of JP-5 in soil from Patuxtent Naval Air Test Center (NATC) under simulated conditions of soil venting. Surfactants and emulsifiers were screened for microbial toxicity and for their capacity to solubilize jet fuel from soil. Three surfactants were subsequently evaluated in 60-day flask aerobic biodegradation experiments. One surfactant was tested in soil columns under simulated soil venting conditions for 47 days. The results of the soil column study showed that the surfactant plus soil venting failed to enhance biodegradation of JP-5 compared to soil venting alone. Soil venting appears to overcome oxygen limitations in unsaturated soil and should be considered for enhanced biodegradation and soil bioremediation at NATC.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Surfactants, emulsifiers, bioremediation, soil, water, fuels, soil venting	24
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

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FINAL REPORT

on

EVALUATION OF INNOVATIVE APPROACHES TO STIMULATE DEGRADATION OF JET FUELS IN SUBSOILS AND GROUNDWATER

to

NAVAL CIVIL ENGINEERING LABORATORY
Code L71
Port Hueneme, California 93043-2693

from

BATTELLE
Columbus Division

August 25, 1989

INTRODUCTION

The Naval Civil Engineering Laboratory (NCEL) is coordinating the decontamination of soils at the fuel farm of Patuxent Naval Air Test Center (NATC), Patuxent River, Maryland. The soils at the fuel farm were contaminated with jet fuel (JP-5) in the winter of 1976-1977 when a pipeline connecting underground storage tanks ruptured. Since that time, the fuel has moved through the sandy soils at the site. Currently, several acres of soil to a depth of approximately 20 to 30 feet, as well as surface waters, are contaminated with jet fuel. Concern is increasing over the extent of fuel contamination and the protection of groundwater in the area. Other hydrocarbons, such as JP-4, also exist at the site.

NCEL is investigating options to cost-effectively decontaminate the vadose (unsaturated) zone in situ. By decontaminating the site, NCEL hopes to prevent future contamination of surface and groundwater. A potentially cost-effective method for in situ soil decontamination is the microbially-mediated biodegradation of fuel hydrocarbons, that is, bioremediation. This process is an attempt to stimulate the

microorganisms that are indigenous to the soil to metabolize fuel hydrocarbons in situ. While most soils contain microorganisms that are capable of degrading hydrocarbons in situ, the factors that limit the bioremediation process need to be overcome. These factors may include restricted bioavailability of the contaminant, nutrient limitations, potential toxicity of fuel hydrocarbons and associated contaminants, inadequate reduction/oxidation (redox) potential, inadequate or excessive moisture, acidic or basic conditions, and oxygen deficiency.

SCOPE AND OBJECTIVES

The objective of this research was to evaluate innovative approaches for stimulating the degradation of jet fuels in soils and potentially groundwater. The innovative approaches evaluated included the use of selected surfactants and emulsifiers to enhance the bioavailability of and thus the biodegradation of jet fuels in contaminated soil collected from the vadose zone of the NATC fuel farm. Aerobic biodegradation experiments with surfactant-amended soils were conducted in the laboratory using both flask and soil column systems. Sterile controls were included to attempt to differentiate between biological and physical-chemical degradation of jet fuel in the soils.

A secondary experiment was conducted to determine the efficiency of air-stripping for the removal of volatile fuel contaminants from groundwater samples collected from NATC. The results of the air-stripping experiment were reported previously; the previous report is included in the current report as Appendix A.

The results of this project should be applicable to future NCEL plans to implement in situ remediation at NATC, either through the use of biodegradation, physical (air-

stripping) removal of volatiles, or a combination of biological and physical techniques.

MATERIALS AND METHODS

SOIL

The soil used in these experiments was collected from NATC fuel farm wells 24 and 25 by personnel from IT Corporation. Cuttings from the drilling of wells 24 and 25 were placed in plastic-lined drums that were transported to Battelle's West Jefferson Laboratory and stored at 4°C. Field-moist soil samples were analyzed for microbial enumeration by dilution plating on nutrient agar.

Representative samples of soil 24 and 25 also were analyzed for fuel hydrocarbon content as follows. Thirty grams of soil were placed in a 250 ml flask and extracted with 100 ml of acetone by orbital shaking for 30 minutes. The supernatant was removed from the soil and 30 ml of the supernatant were diluted to 100 ml with distilled water. The final volume (100 ml) was passed through a preconditioned C_{18} prep-sep column under vacuum. The prep-sep columns were preconditioned with 1 to 2 ml of methanol and distilled water. After the acetone-water soil extracts were passed through the prep-sep columns, the columns were eluted with 2 ml of methylene chloride. One microliter of the methylene chloride eluates was then analyzed on a Varian 3700 gas chromatograph (GC) equipped with a flame-ionization detector (FID) using JP-4 as standards. The GC conditions were as follows:

Column:	6 ft. x 2 mm I.D. 3% OV 101
Gas flow rate:	20 ml/minute N_2
Injector temperature:	60°C
Detector temperature:	300°C

Column temperature: 40°C for 4 minutes, increasing at 10°C/minute up to a final temperature of 250°C, and held at the final temperature for 4 minutes.

Peaks were integrated with a Varian 4270 integrator and compared to standards of jet fuel.

SURFACTANT SELECTION

The objective of this task was to identify surfactants and emulsifiers that effectively solubilized jet fuel when added to soil at microbially non-toxic concentrations. Fifty-three different surfactants and emulsifiers were tested for their use in enhancing biodegradation of jet fuel in soil. The surfactants and emulsifiers initially were identified based on recommendations from major manufacturers and through the surfactant/emulsifier literature. The surfactants and emulsifiers then were screened for their toxicity to microorganisms (using a Microtox bioassay system). Non-inhibitory materials then were screened for their ability to emulsify and release jet fuel from soil at microbially non-toxic concentrations as follows.

To screen each surfactant or emulsifier, its ability to extract jet fuel from soil was compared to both water and acetone. For water extractions, 10 g of soil 24 (which proved to be the most contaminated soil based on the GC analysis described above) were combined with 20 ml of distilled water in a 40-ml test tube. The soil and water were vortexed for 5 minutes and the supernatant was separated from the soil by filtration through #1 Whatman filter paper. The supernatant was diluted to 100 ml with distilled water and then passed through a C_{18} prep-sep column (conditioned with methanol and water). The column was then eluted with 2 ml of methylene chloride for FID-GC analysis.

Acetone extractions were done similarly to the water extractions. Twenty milliliters of acetone were added to 10 g of soil 24 and vortexed for 5 minutes. The slurry was then filtered, the filtrate was diluted as before to 100 ml with distilled water, passed over a preconditioned C_{18} prep-sep column, and eluted with 2 ml of methylene chloride for FID-GC analysis.

Finally, the surfactant and emulsifier extractions of soil 24 were done similarly. Twenty milliliters of the appropriate concentration of each surfactant or emulsifier were vortexed for 5 minutes with 10 g of soil 24, filtered or centrifuged, diluted to volume with distilled water, passed through a prep-sep column, extracted with methylene chloride, and analyzed for hydrocarbons by FID-GC.

Thus, the capacity of non-toxic concentrations of each surfactant or emulsifier to extract jet fuel from soil 24 was compared to extractions with both water (presumably little or no extracting capacity) and acetone (presumably nearly 100 percent extracting capacity). The assumption was that the ability to solubilize jet fuel from soil is related to enhancing the bioavailability of the jet fuel to soil microorganisms. As a result of this screening, three surfactants were selected for preliminary flask studies of jet fuel biodegradation in soil 24, as described below.

PRELIMINARY FLASK STUDIES

A flask study was setup to preliminarily evaluate the utility of three surfactants--numbers 21, 39, and 49--to enhance jet fuel biodegradation in contaminated soil 24. Surfactants 21, 39, and 49 were GAF Emulphor ON-870, Thompson/Hayward T-Det N-95, and Texaco Surfonic N-95, respectively. The factorial experimental design included two concentrations of each of the three surfactants (in triplicate replication) in 250-ml biometer

flasks containing 100 g (on a dry-weight basis) of [illegible] 24. Each biometer flask was equipped with a side-arm tube containing 10 ml of 0.6 N NaOH to trap evolved CO2. The surfactant concentrations included 0.5 and 1.0 percent (v/w, surfactant/soil). The soil in all flasks was amended with Restore 375 at the recommended rate of 2 g/100 g soil. The moisture content of the soils was maintained at 60 percent of field capacity and the flasks were incubated aerobically in the dark at 23°C. A sterile control (one replicate) was included for each surfactant. The sterile controls were obtained by the addition of 500 µg/g Cd (as $CdCl_2$) and 500 µg/g Hg (as $HgCl_2$) to biometer flasks containing nutrient-amended soil 24 and surfactant. Moisture was maintained at approximately 60 percent of field capacity in all flasks by the weekly addition of water (sterilized water for the sterile controls), if necessary.

Biodegradation was monitored by measuring the cumulative evolution of CO_2 from each flask over a 60-day incubation period. Also, at days 0, 30, and 60, appropriate flasks were sacrificed and the soils were analyzed for fuel hydrocarbon concentrations and microbial enumeration. Cumulative CO_2 was measured by weekly sampling and recharging NaOH traps. The NaOH removed from the biometer flask at each sampling was combined with 5 ml of 1.3 N $BaCl_2$ to precipitate absorbed CO_2 as $BaCO_3$. Evolved CO_2 was determined by titration of unreacted NaOH against standardized HCl, using a Fisher Automatic Titrator-II.

The jet fuel concentration in flask at days 0, 30, and 60 was determined by a dual extraction of 10-g aliquots of each soil, using water followed by acetone. The extraction and FID-GC procedures were as described previously, except that a Hewlett-Packard 5890 gas chromatograph with FID was used. Microbial enumeration by the soil dilution plating technique was carried out on both mineral salts agar containing a JP-4-saturated filter paper taped to the petri dish lid, and nutrient agar.

The results of the preliminary flask study led to the identification of surfactant number 21 as a likely candidate for scale-up to soil column studies. Thus, soil columns containing fuel-dosed soil 24 dosed with surfactant 21 were setup, as described below.

SOIL COLUMN DESIGN AND TREATMENT SYSTEM

Soil 24 was amended with Restore 375 (2 g/100 g soil) and JP-5 to obtain a final fuel concentration of 150 µg/g. Fifteen, 12 inch x 1.5 inch glass columns containing 250 g (dry weight basis) of fuel-dosed soil 24, and three empty (method blank) columns, were placed vertically in a specially designed wooden rack. The ends of each column were sealed with one-hole rubber stoppers. The bottom stopper (air inlet) contained a fritted glass tube to aid in gas dispersion; the fritted tube was packed 2 to 3 inches deep with glass wool to prevent breakage or plugging. The bottom stopper of each tube was connected to a manifold that delivered CO_2-free air. That is, incoming room air passed through an activated carbon filter, then through a backflush trap, then through a solution of 1 N NaOH (to remove background CO_2), and finally into a stainless steel manifold with an independent connection to each soil column. The top stopper (air outlet) of each column was connected via tubing to a CO_2-trap (1 N NaOH, to capture microbially evolved CO_2 from the soil columns), which in turn was connected to two in-line Orbo tubes (Supelco) for trapping organic volatiles, and finally to a multi-channel peristaltic pump. Thus, each column was independently aerated at a controlled rate, and evolved CO_2 and organic volatiles were trapped from each column.

The experimental design included two concentrations of surfactant 21--0.5 and 0.2 percent (w/w, surfactant/soil)--in triplicate. Control columns included three 0-dose soils (that is, no surfactant), and triplicate sterile controls for each

surfactant concentration. The sterile controls were obtained by dosing the soils with 500 µg/g Cd (as $CdCl_2$) and 500 µg/g Hg (as $HgCl_2$). Moisture was gravimetrically maintained at approximately 50 percent of field capacity in by the addition of water (sterilized water for the sterile controls), if necessary.

The cumulative evolution of CO_2 from each column was determined over a 47-day incubation period. Cumulative CO_2 was measured by weekly sampling and recharging NaOH traps, as described previously. At day 0, the soils were analyzed for fuel hydrocarbon determination and microbial enumeration as described earlier. At day 47, the columns were sacrificed and the soils were again analyzed for fuel hydrocarbon concentrations and microbial enumeration.

RESULTS AND DISCUSSION

SURFACTANT SELECTION

One of the factors that appears to limit the biodegradation process in soils is that the target compound may be inaccessible (not bioavailable) due to adsorption or is coated with impenetrable materials (Hill, 1978; Knezovich et al., 1987). Surfactants and emulsifiers have been used with mixed success to attempt to enhance the bioavailability of recalcitrant compounds (Urano and Saito, 1985). Thus, the objective of the surfactant screening task was to identify surfactants and emulsifiers that would enhance the bioavailability of fuel hydrocarbons in soil, yet would not inhibit soil microbial activity. The identification of a cost-effective method for improving the bioavailability of fuel hydrocarbons in soil could then be scaled up and evaluated in flask, column, and eventually field studies for in situ bioremediation.

The surfactant screening revealed that most of the surfactants and emulsifiers examined were either microbially

toxic or ineffective at solubilizing fuel hydrocarbons from soil. A number of surfactants and emulsifiers were evaluated for their microbial toxicity (Table 1). Those exhibiting relatively low toxicity in the Microtox Bioassay system were screened further for their ability to solubilize JP-5 from soil, relative to acetone.

Based on the initial screening, three surfactants or emulsifiers were chosen for flask studies. These included surfactants/emulsifiers numbers 21, 39, and 49. These three surfactants were chosen because of their combined low or non-toxic nature as determined by the Microtox bioassay, and their ability to solubilize fuel hydrocarbons from soil. The gamma values in Table 1 are relative values of toxicity to Photobacterium phosphoreum of the Microtox bioassay; the higher the value, the more toxic the test material. The gamma value for surfactant 21, 39, and 49 when tested at concentrations of 0.45 percent (v/v) were 0.04, 0, and 0, respectively. On the other hand, some materials, such as surfactants 22 and 53 at the same test concentrations, were highly toxic and gave gamma values of 14.2 and 31.1, respectively.

Materials 21, 39, and 49 also proved to be relatively effective at solubilizing JP-5 in soil. Surfactant 49, an alkylphenol ethoxylate, at a 1 percent (v/w) concentration was the most effective surfactant tested for extracting jet fuel from soil; its value relative to acetone (i.e., 100 percent) was 137 percent. Surfactant 49 at a 0.5 percent (v/w) concentration also was relatively effective at solubilizing jet fuel from soil; its value relative to acetone was 108 percent. Surfactant 39 was a nonylphenol ethoxylate and at 0.5 and 1 percent (v/w) yielded values of 108 and 93 percent, respectively, relative to acetone. Finally, surfactant 21, a polyoxyethylated oleyl alcohol, at 0.5 and 1 percent (v/w) concentrations was 97 and 81 percent, respectively, as effective as acetone.

TABLE 1. SURFACTANTS AND EMULSIFIERS EVALUATED FOR THEIR UTILITY IN SOIL BIODEGRADATION EXPERIMENTS.

Number	Surfactant	Gamma[1]	Solubility Capacity Relative to Acetone (Percent) Concentration 0.5%	1.0%
1	Shell Neodol 23-6.5T	1.63	nd[2]	nd
2	Shell Neodol 23-6.5	7.91	nd	nd
3	Shell Neodol 25-9	3.04	nd	nd
4	Shell Neodol 91-2.5	--[3]	nd	nd
5	Shell Neodol 91-6	--	nd	nd
6	Shell Neodol 91-8	--	nd	nd
7	Milliken SynFac 222	0.18	59.3	97.3
8	Milliken SynFac 334	0.16	33.6	89.5
9	Milliken SynFac 334-13	0	9.9	9.9
10	Milliken SynFac 8210	0.48	29.9	131.0
11	Milliken SynFac 8216	0	63.9	71.3
12	Henkel Nopalcol 2-OL	16.12	nd	nd
13	Henkel Nopalcol 4-L	1.23	nd	nd
14	Henkel Nopalcol 6-L	--	nd	nd
15	ICI Ahcowet RS	--	nd	nd
16	ICI Tween 20	8.73	nd	nd
17	ICI Tween 80	1.12	nd	nd
18	AET Land Reclaimer	--	27.4	46.6
19	NL Aktaflo-E	2.87	nd	nd
20	Norman Fox Norfox	--	nd	nd
21	GAF Emulphor ON-870	0.04	114.0	97.5
22	GAF Emulphogene BC 420	14.22	nd	nd
23	GAF Igepal CO-520	0.08	15.9	85.0
24	GAF Peganate L-20	3.22	nd	nd
25	Witco Witcomul 4143	--	nd	nd
26	Witco Witcomul 4144	--	nd	nd
27	Witco Witconate P-1059	8.73	nd	nd
28	Witco Witconate AOS	4.82	nd	nd
29	Mazer Chem Mazon 1086	1.54	nd	nd
30	Mazer Chem S-Maz 20	--	nd	nd
31	Mazer Chem S-Maz 80	--	nd	nd
32	Mazer Chem S-Maz 85	2.87	nd	nd
33	Mazer Chem T-Maz 20	2.14	nd	nd
34	Mazer Chem T-Maz 80	1.18	nd	nd
35	Mazer Chem T-Maz 85	1.37	nd	nd
36	Mazer Chem Mazawet 77	--	nd	nd
37	Thompson-Hayward T-Det N6	0	48.8	93.9

TABLE 1 (CONTINUED)

38	Thompson-Hayward T-Det N8	0	18.4	52.9
39	Thompson-Hayward T-Det N9.5	0	127.6	109.6
40	Henkel Agrimul JI	0.82	nd	nd
41	Henkel Agrimul 26-B	0.71	26.7	29.3
42	Henkel Agrimul Nopalco 4-O	2.19	nd	nd
43	Henkel Agrimul Nopalco 6-O	3.60	nd	nd
44	Lipo Chem Lipocol L-4	--	nd	nd
45	Lipo Chem Lipocol L-12	4.89	nd	nd
46	Lipo Chem Lipocol L-23	0.56	31.6	26.9
47	Lipo Chem Lipocol TD-12	1.57	nd	nd
48	Texaco Surfonic N-40	--	nd	nd
49	Texaco Surfonic N-95	0	122.5	153.4
50	Rohm and Haas Triton N-60	0.09	39.5	77.7
51	Rohm and Haas Triton N-57	0.25	42.0	70.1
52	Rohm and Haas Triton N-35	--	nd	nd
53	Rohm and Haas Triton N-45	31.13	nd	nd

(1) Gamma is a relative toxicity value indicating the inhibition of light output by the test bacterium, Photobacterium phosphoreum; the higher the value, the greater the toxicity.

(2) nd = not determined because of the relatively high toxicity of the material.

(3) -- = not determined because of solubility problems.

Therefore, materials 21, 39, and 49 were tested in flask studies to further screen for their microbial toxicity and their ability to solubilize fuel hydrocarbons in soil.

PRELIMINARY FLASK STUDIES

The objective of the flask studies was to test the materials identified in the surfactant screening task for their utility in biodegradation experiments with fuel-contaminated soil from NATC. Three endpoints were evaluated to assess the usefulness of surfactants 21, 39, and 49: cumulative evolution of CO_2 from surfactant-amended contaminated soil through 60 days of incubation; changes in JP-5 concentrations in the surfactant-amended soils through the incubation period; and changes in numbers of total heterotrophic and hydrocarbon-degrading bacteria in the surfactant-amended soils. Based on the results of the flask studies, one surfactant would be used in subsequent soil column experiments.

Two concentrations for each of the three surfactants were tested in the CO_2 evolution experiments. High and low concentrations were 1.0 and 0.5 percent (v/w), respectively. The high concentration of surfactant 21 resulted in the greatest evolution of CO_2-C, 47 mg, among all treatments over the 60-day aerobic incubation (Figure 1). This was followed in order by surfactant 49 high (20 mg CO_2-C evolved); surfactant 49 low (18 mg CO_2-C evolved); surfactant 39 high (15 mg CO_2-C evolved); and surfactants 39 high and 21 low (15 mg CO_2-C evolution). Sterile and 0-dose controls were included for all treatments and evolved approximately 10 mg of CO_2-C over the 60-day aerobic incubation.

These results indicate either that surfactant 21 enhanced the bioavailability and biodegradation of fuel hydrocarbons in soil, or that it was itself biodegraded to CO_2-C. Thus, at days 0, 30, and 60 during the 60-day aerobic incubation, soil samples from the flask experiment were extracted with both

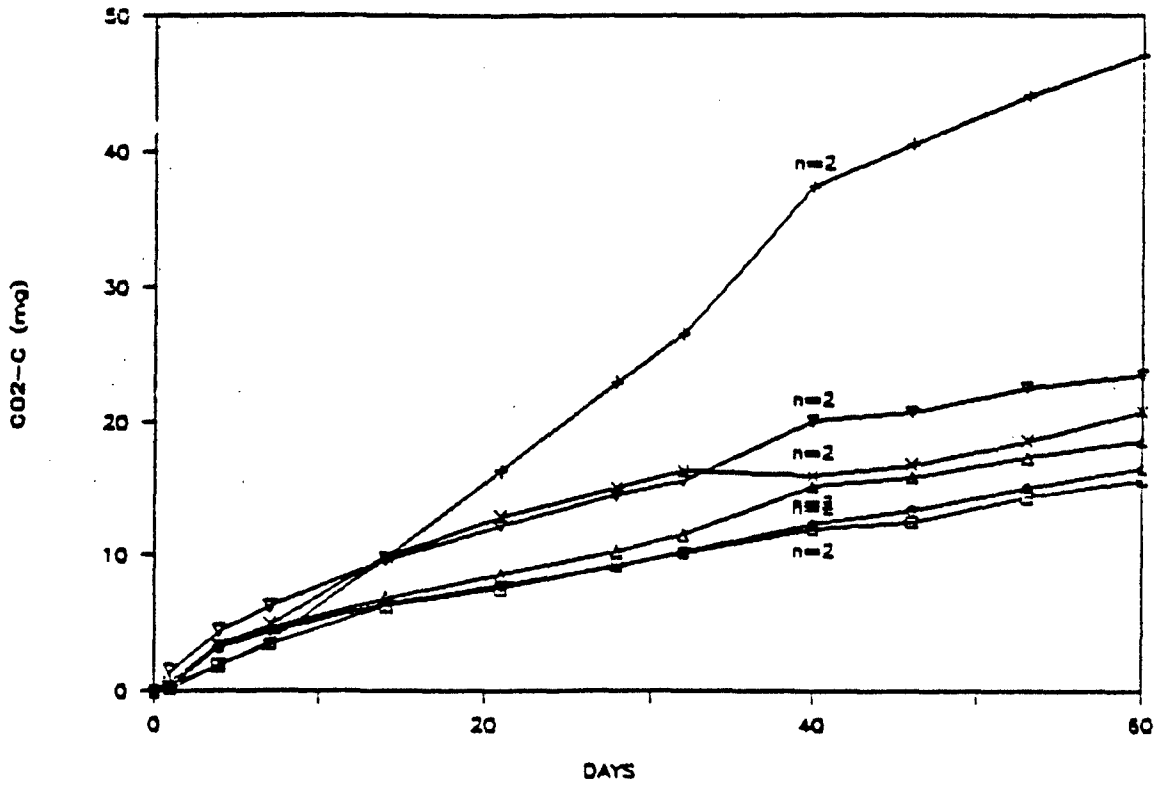

FIGURE 1. CUMULATIVE CO_2 EVOLUTION THROUGH 60 DAYS FROM NATC SOIL 24 AMENDED WITH SURFACTANTS 21, 39, AND 49: SQUARE, 21 LOW; PLUS, 21 HIGH; DIAMOND, 39 LOW; TRIANGLE, 39 HIGH; X, 49 LOW; AND INVERTED TRIANGLE, 49 HIGH.

water and acetone and the extracts analyzed for JP-5. The results (Figure 2) include the amount of JP-5 extracted from each soil using both water and acetone, as described in the Materials and Methods section. The results indicate a loss of hydrocarbons through time in the high surfactant treatments compared to the low surfactant treatments and no-surfactant controls. However, decreases in JP-5 concentrations occurred in sterile soils with high surfactant concentrations as well as microbially active soils (Figure 2.c). The sterility of these soils was confirmed by plating soil samples for microbial enumeration. Thus, the loss in JP-5 from microbially active and sterile soils receiving high concentrations of surfactants may have been due to volatilization or some other physical or chemical process, instead of biodegradation.

The soils from the CO_2 evolution flask experiment were enumerated on both nutrient agar and mineral salts agar in which jet fuel was the only carbon source. The results for the nutrient agar plating (Figure 3.a) show a general increase in microorganisms from day 0 through the 30- and 60-day samplings. The greatest increase in microbial numbers occurred with the high concentration of surfactant 21; at days 30 and 60, 9.51×10^7 and 7.28×10^7 colony forming units per gram of soil (CFU/g), respectively, were enumerated on nutrient agar. All other enumeration results for days 30 and 60 were an order of magnitude less than for the high dose of surfactant 21. Similar results were obtained soil plating on mineral salt agar plus jet fuel (Figure 3.b).

These enumeration results correlate with the evolution of CO_2 in the flask studies (see Figure 1). Thus, the increased CO_2 evolution from soil treated with surfactant 21 appears to be the result of increased numbers and metabolic activity of microorganisms. However, since the loss of JP-5 was comparable in <u>all</u> soils treated with high surfactant concentrations (see Figure 2), then the enhanced microbial activity in soil

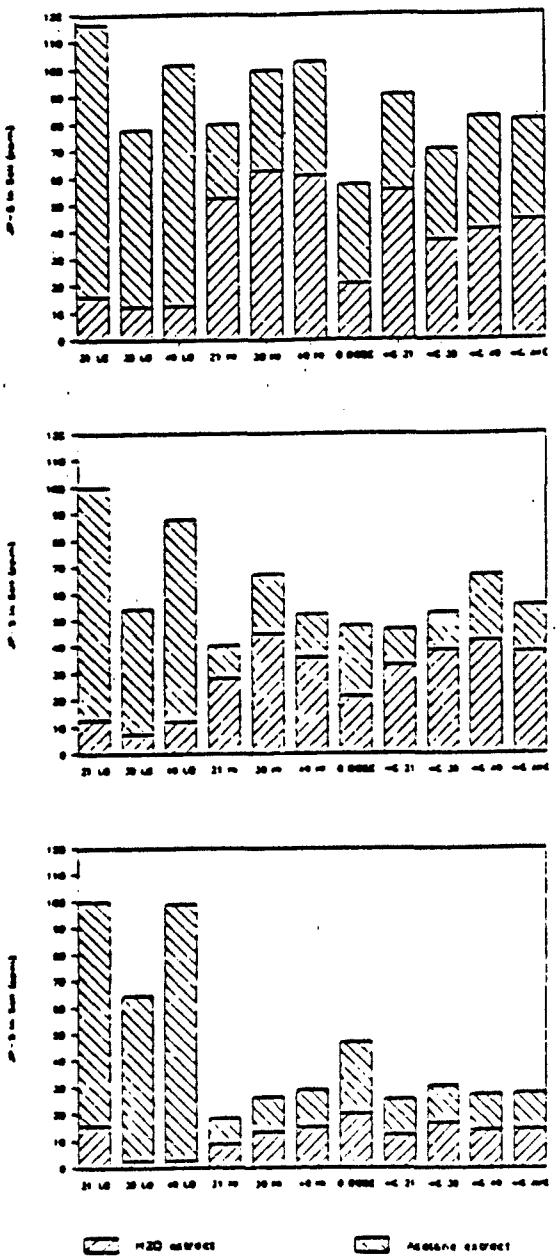

FIGURE 2. JP-5 EXTRACTED FROM NATC SURFACTANT-AMENDED SOIL 24 USING WATER FOLLOWED BY ACETONE. A. DAY 0; B. DAY 32; C. DAY 60.

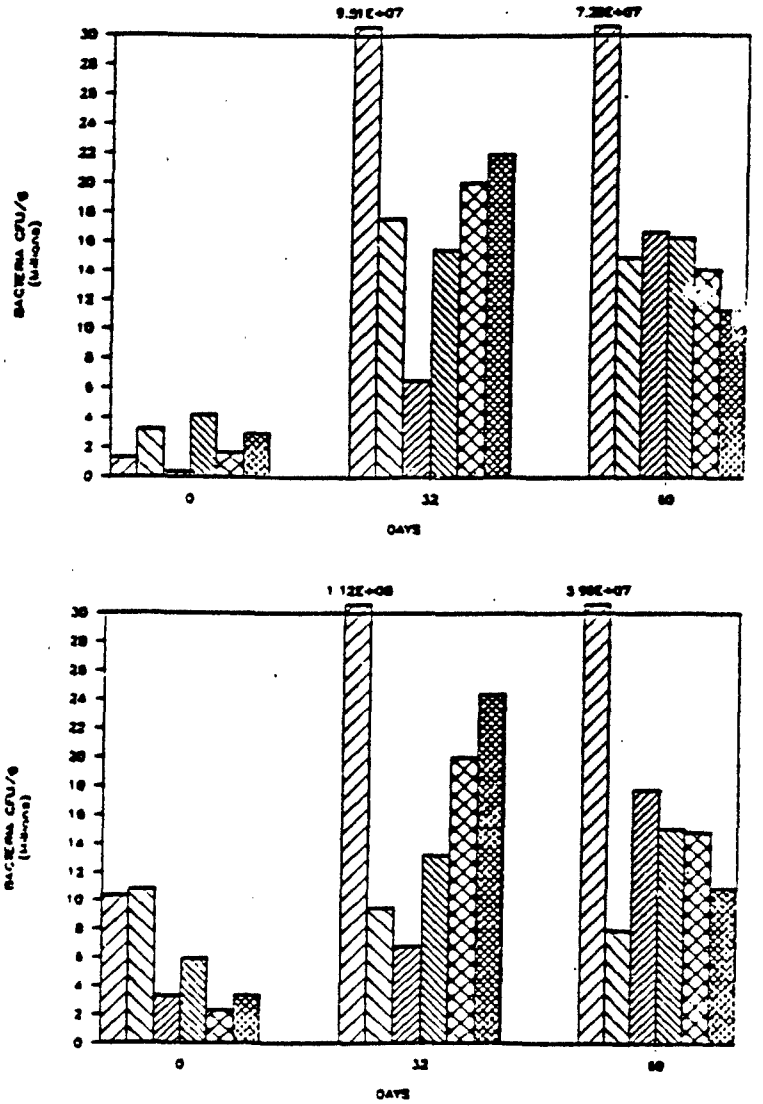

FIGURE 3. MICROBIAL ENUMERATION OF FLASK SOILS AT DAYS 0, 32, AND 60. A. NUTRIENT AGAR: READING FROM LEFT TO RIGHT, THE BARS REPRESENT SURFACTANTS 21 HIGH, 21 LOW, 39 HIGH, 39 LOW, 49 LOW, AND 49 HIGH. B. MINERAL SALTS AGAR: FORM LEFT TO RIGHT THE BARS REPRESENT 21 HIGH, 21 LOW, 39 HIGH, 39 LOW, 49 HIGH, AND 49 LOW.

containing the high concentration of surfactant 21 may not have led to increased JP-5 biodegradation. This was unexpected since increased microbial activity theoretically should result in fuel hydrocarbon degradation by cometabolism if not by direct metabolism. Therefore, to attempt to evaluate further the potential use of surfactant 21 in enhanced biodegradation, it was applied to NATC fuel-contaminated soil in aerobically incubated soil columns.

SOIL COLUMN STUDIES

The soil column experiment was designed to simulate what should occur in the field under a soil venting situation. That is, in situ fuel biodegradation in subsurface soils tends to be limited by the supply of oxygen (API, 1987) and one mechanism to supply oxygen is through soil venting. Thus, the soil column experiment combined aeration through soil venting with the addition of a surfactant to enhance the solubility of fuel hydrocarbons in soil. Under these conditions, any loss of fuel hydrocarbons could be due to enhanced volatilization and/or enhanced biodegradation.

Cumulative evolution of CO_2-C was determined over 47 days of aerobic incubation of the soil columns containing nutrient-amended, JP-5-contaminated soil plus high (1.0 percent, v/w) and low (0.5 percent, v/w) concentrations of surfactant 21. Sterile controls were included that initially were sterilized with Cd and Hg as described in the Materials and Methods section. However, the sterile controls became contaminated during the incubation and thus were autoclaved. As a result, the sterile controls were useful for the CO_2 evolution experiment but not for the determination of residual fuel hydrocarbons by extraction of the soils because of the potential loss of JP-5 by volatilization during the autoclaving process.

Unexpectedly, soil columns containing both high and low concentrations of surfactant 21 evolved nearly the same amount of CO_2-C as the no-surfactant control soil columns (Figure 4). That is, after 47 days of aerobic incubation, the amount of CO_2-C evolved from the no-surfactant control, surfactant 21-high, and surfactant 21-low soil columns wa. 19.9 ± 1.26, 21.4 ± 6.16, and 20.4 ± 5.18 mg CO_2-C, respectively. In comparison, the surfactant 21-high and 21-low sterile control columns evolved 7.99 ± 2.86 and 10.7 ± 5.97 mg CO_2-C, respectively. Thus, unlike in the flask experiment discussed in the previous section, the addition of surfactant 21 to nutrient-amended, fuel-contaminated soil did not appear to stimulate microbial activity compared to the no-surfactant control soil.

To evaluate further the actions of surfactant 21 in the soil columns, soil samples from each column were extracted at the conclusion of the 47-day aerobic incubation, as described in the Materials and Methods section. The mean recovery of JP-5 from the triplicate soil columns containing the high and low concentrations of surfactant 21 was 138 ± 9.7 and 113 ± 27 µg/g, respectively (Figure 5). In comparison, the no-surfactant control soil columns contained 21 ± 4.9 µg/g of JP-5 after the 47 days of soil venting.

The interpretation of these results is not clear because of the similar amounts of CO_2-C evolved from the surfactant and no-surfactant soil columns, as discussed above. That is, if the loss of JP-5 from the no-surfactant control soil columns had correlated with increased CO_2 evolution relative to the surfactant-treated soils, then a conclusion might be that surfactant 21 actually inhibited the biodegradation of JP-5. Another possible interpretation of the results is that surfactant 21 did indeed inhibit the biodegradation of JP-5 in the soil by serving as a preferred (or more bioavailable) carbon source. If true, then the evolution of CO_2 from surfactant-treated soil columns could be the result of the biodegradation of the

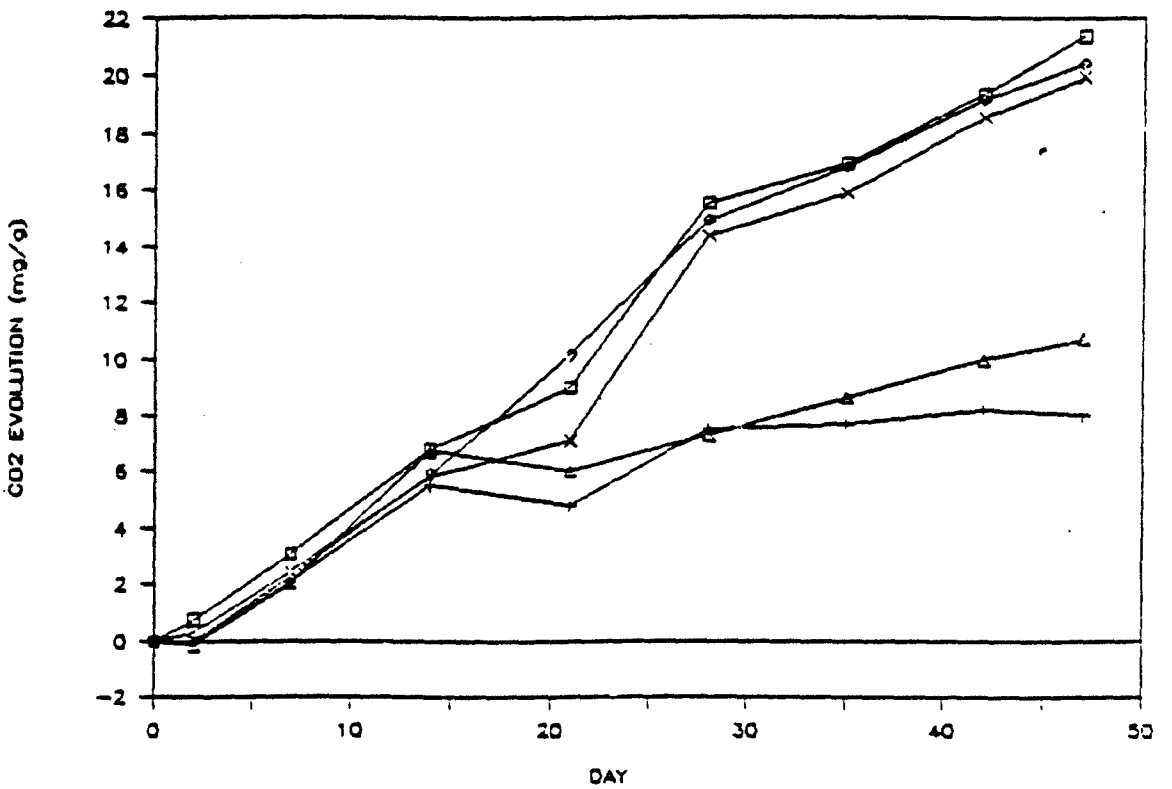

FIGURE 4. CUMULATIVE CO_2 EVOLUTION THROUGH 47 DAYS FROM SOIL COLUMNS AMENDED WITH SURFACTANT 21: SQUARE, 21 HIGH; PLUS, 21 HIGH STERILE; DIAMOND, 21 LOW; TRIANGLE, 21 LOW STERILE; X, NO SURFACTANT.

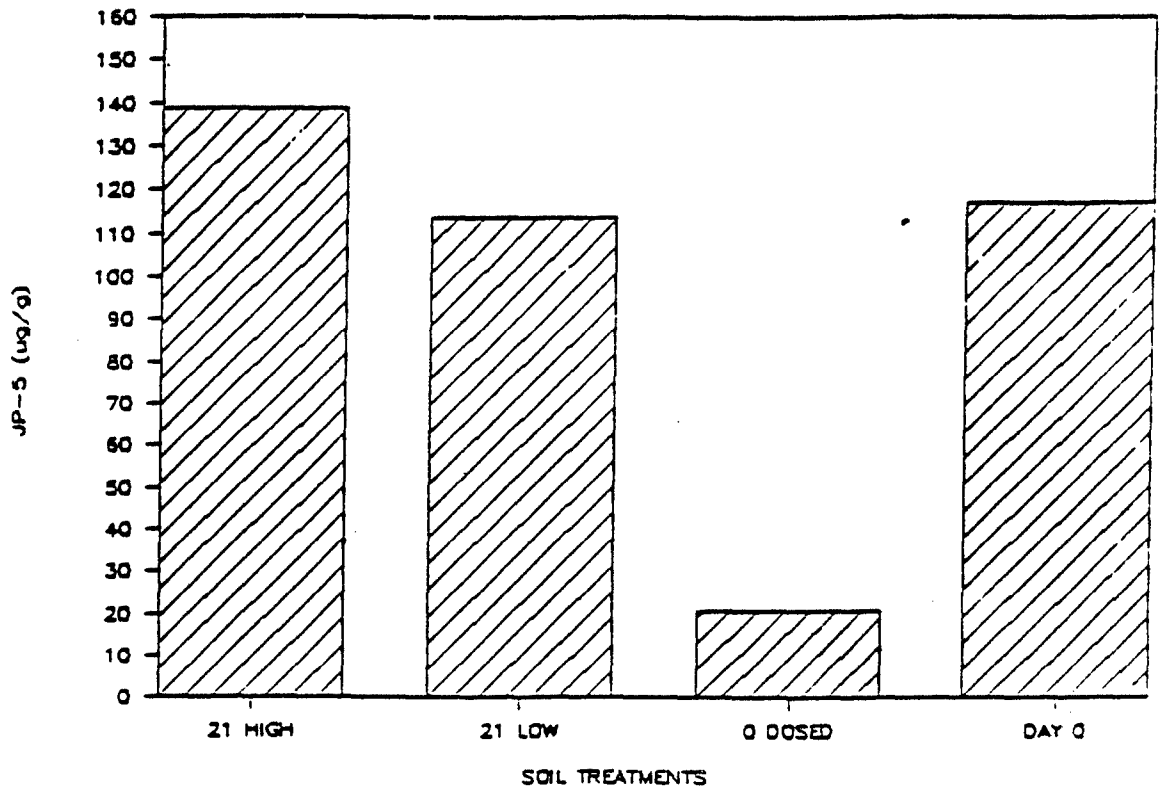

FIGURE 5. RECOVERY OF JP-5 FROM SOIL COLUMNS AFTER 47 DAYS OF AEROBIC INCUBATION WITH OR WITHOUT SURFACTANT 21.

surfactant itself, while CO_2 from the no-surfactant control columns resulted from JP-5 biodegradation.

The interpretation of the CO_2 evolution and soil JP-5 data is complicated further by the results of microbial enumeration in the soil columns. As in the flask study, surfactant 21 appeared to stimulate numbers of microorganisms on both nutrient agar and mineral salts agar (Figure 6). For example, the numbers of nutrient agar platable bacteria in the presence of high and low concentrations of surfactant 21 were $11.8 \times 10^6 \pm 3.33 \times 10^6$ and $10.1 \times 10^6 \pm 2.09 \times 10^6$ CFU/g, respectively. The no-surfactant control, on the other hand, yielded $2.79 \times 10^6 \pm 3.18 \times 10^6$ CFU/g. Similarly, the numbers of organisms recovered from soil dosed with high and low concentrations of surfactant 21 and plated on mineral salts agar plus JP-5 were $3.69 \times 10^6 \pm 1.08 \times 10^6$ and $2.48 \times 10^6 \pm 0.81 \times 10^6$ CFU/g, respectively. In the case of mineral salts agar, the no-surfactant control yielded $0.86 \times 10^6 \pm 0.69 \times 10^6$ CFU/g. However, the increased numbers of organisms in the presence of surfactant 21 did not lead to increased evolution of CO_2 (see Figure 4) or to increased loss of JP-5 (see Figure 5).

CONCLUSIONS

The objective of this study was to evaluate the feasibility of surfactant-enhanced biodegradation of JP-5 in soil under simulated conditions of soil venting. One of the criteria for demonstrating biodegradation is the simultaneous loss of parent material and increase in cell biomass (Healy and Daughton, 1986). While increases in microbial numbers were obtained in the presence of surfactant 21 in the soil column experiment, no loss of parent material (JP-5) was observed. Thus, surfactant 21 did not appear to enhance the biodegradation of JP-5 in soil column studies. This was unexpected based on the flask studies and the surfactant/emulsifier screening conducted prior to the soil

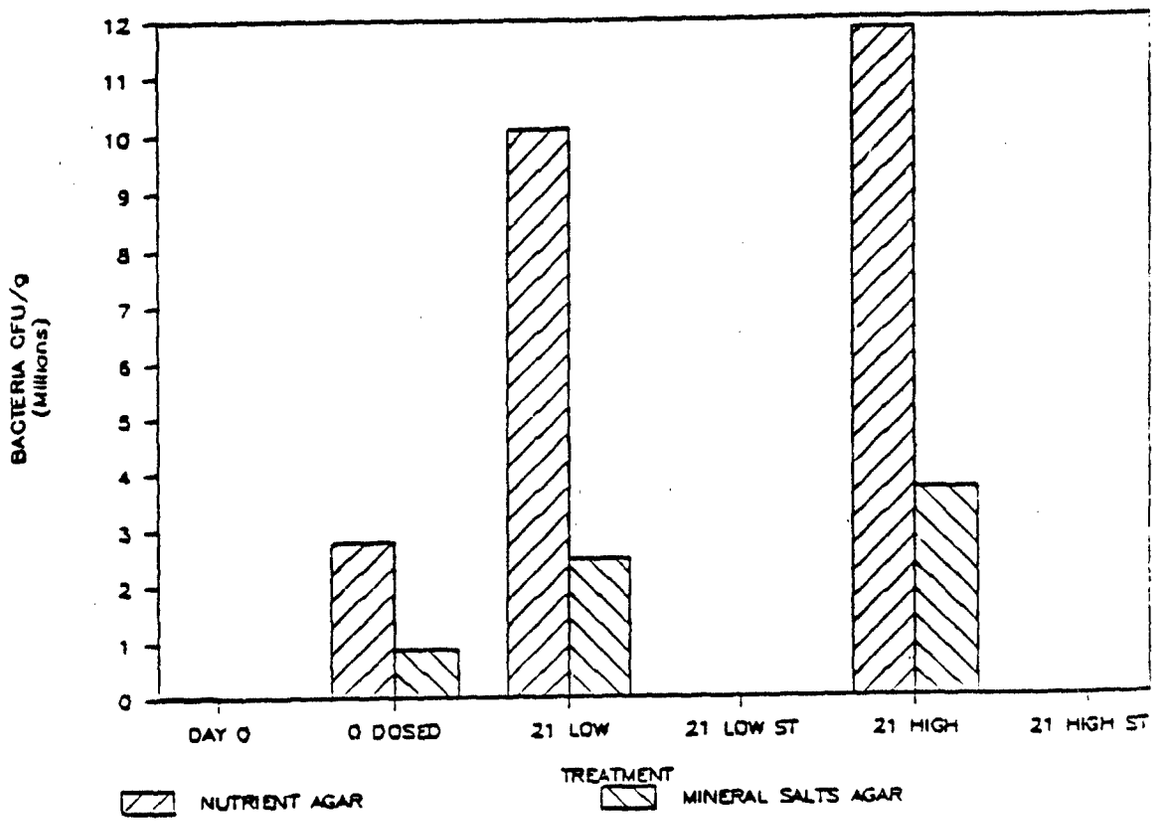

FIGURE 6. MICROBIAL ENUMERATION ON NUTRIENT AGAR AND MINERAL SALTS AGAR OF SOIL FROM SOIL COLUMNS AFTER 47 DAYS OF AEROBIC INCUBATION.

column studies. The reason that surfactant 21 failed to stimulate JP-5 biodegradation is not clear from the results of the study. However, the possibility exists that surfactant 21 provided an alternative and preferred carbon source for microbial metabolism, and thus inhibited the biodegradation of JP-5. Even under these conditions, however, decreases in JP-5 due to cometabolism should have occurred. The results of this study suggest that surfactant-enhanced biodegradation may not be a feasible option for in situ bioremediation, or that a more intensive screening process should be used to identify appropriate surfactants.

Nevertheless, the results suggest that soil venting--the process of aerating soils by pumping air through the soil profile--offers an effective means for enhancing fuel biodegradation in the vadose zone. This conclusion is based on the decrease of JP-5 in no-surfactant control soil columns from approximately 150 µg/g on day 0 to approximately 20 µg/g after 47 days of soil venting. These results agree with conclusions of others (e.g., API, 1987) that oxygen (or some other electron acceptor) is the limiting factor for in situ biodegradation.

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